Folate–vitamin B-12 interaction in relation to cognitive impairment, anemia, and biochemical indicators of vitamin B-12 deficiency[1–5]

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ABSTRACT

Previous reports on pernicious anemia treatment suggested that high folic acid intake adversely influences the natural history of vitamin B-12 deficiency, which affects many elderly individuals. However, experimental investigation of this hypothesis is unethical, and the few existing observational data are inconclusive. With the use of data from the 1999–2002 National Health and Nutrition Examination Survey (NHANES), we evaluated the interaction between high serum folate and low vitamin B-12 status [ie, plasma vitamin B-12 $<$ 148 pmol/L or methylmalonic acid (MMA) $>$ 210 nmol/L] with respect to anemia and cognitive impairment. With subjects having both plasma folate $\leq$ 59 nmol/L and normal vitamin B-12 status as the referent category, odds ratios for the prevalence of anemia compared with normal hemoglobin concentration and impaired compared with unimpaired cognitive function were 2.1 (95% CI: 1.1, 3.7) and 1.7 (95% CI: 1.01, 2.9), respectively, for those with low vitamin B-12 status but normal serum folate and 4.9 (95% CI: 2.3, 10.6) and 5.0 (95% CI: 2.7, 9.5), respectively, for those with low vitamin B-12 status and plasma folate $>$59 nmol/L. Among subjects with low vitamin B-12 status, mean circulating vitamin B-12 was 228 pmol/L for the normal-folate subgroup and 354 pmol/L for the high-folate subgroup. We subsequently showed increases in circulating homocysteine and MMA concentrations with increasing serum folate among NHANES participants with serum vitamin B-12 $<$ 148 pmol/L, whereas the opposite trends occurred among subjects with serum vitamin B-12 $\geq$ 148 pmol/L. These interactions, which were not seen in NHANES III before fortification, imply that, in vitamin B-12 deficiency, high folate status is associated with impaired activity of the 2 vitamin B-12–dependent enzymes, methionine synthase and MMA–coenzyme A mutase. *Am J Clin Nutr* 2009; 89(suppl):702S–6S.

INTRODUCTION

Folate and vitamin B-12 interrelation

The vitamins folic acid and B-12 serve as coenzymes in one-carbon metabolism. Specifically, a carbon unit from serine or glycine reacts with tetrahydrofolate (THF) to form methylene-THF. This may be used for the synthesis of thymidylate, a DNA nucleotide, or for purine synthesis. Folate deficiency-related macrocytic anemia is due to failure of precursor blood cells to divide because of a lack of DNA. The adverse effect of vitamin B-12 deficiency on DNA synthesis is explained by the ''methylfolate trap hypothesis'' (1). Vitamin B-12 acts as a cofactor for methionine synthase (MS), which catalyzes the remethylation of homocysteine to methionine. The methyl group is donated by methyl-THF, which is derived by the irreversible reduction of methylene-THF to methyl-THF by methylene-THF reductase. If MS is inactivated by a lack of vitamin B-12, the result is a functional folate deficiency (ie, a lack of the nonmethylated folates needed for serine-glycine interconversion and the synthesis of purines and pyrimidines) as folate becomes increasingly ''trapped'' as methyl-THF.

Hyperhomocysteinemia is another consequence of deficiencies of either folate or vitamin B-12 (2). This effect is due, in part, to the requirement by MS for both folate and vitamin B-12. Furthermore, a considerable proportion of the methionine that results from homocysteine remethylation is converted to *S*-adenosylmethionine, which regulates the one-carbon pathway by inhibiting methylene-THF reductase and activating the homocysteine-disposing enzyme, cystathionine-β synthase. *S*-adenosylmethionine also serves as the sole methyl donor for the central nervous system, which may explain associations between folate deficiency and vitamin B-12 deficiency and cognitive impairment and mental illness (3, 4). One of the most devastating consequences of vitamin B-12 deficiency is a classic neuropathy called combined degeneration of the spinal cord (5). The mechanism by which vitamin B-12 deficiency leads to this fatal demyelinating illness is unknown, but its specific link to vitamin B-12 deficiency, but not folate deficiency, may provide a clue to the causal pathway.

Another unique consequence of vitamin B-12 deficiency relates to its role in the isomerization of *L*-methylmalonyl–

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coenzyme A (CoA) to succinyl-CoA—a reaction that, unlike the remethylation of homocysteine, occurs in the mitochondria and does not involve folate. Thus, vitamin B-12 deficiency specifically results in increased methylmalonic acid (MMA) concentrations in both plasma and urine.

Pernicious anemia and excessive intake of folic acid

After studying malnourished pregnant women in India in the late 1920s, Lucy Wills described a macrocytic anemia that shared some features with the blood abnormalities of elderly Europeans with pernicious anemia (6). For example, the cytologic properties of the 2 anemias were identical, and both types responded well to crude liver extract. However, only the tropical illness responded to yeast extract. Furthermore, although purified liver extract had proved effective in the treatment of pernicious anemia, it did not cure the tropical form (6). This difference in response between the 2 anemias led to the suggestion that the ''Wills factor'' in the yeast extract was distinct from the ''extrinsic factor'' in the purified liver extract. This hypothesis was subsequently verified. However, the identification of vitamin B-12 as the anti–pernicious anemia factor in liver (7) followed the isolation of folic acid from yeast (8) by several years, during which time folic acid was administered in large doses to patients with pernicious anemia (9). Because the inappropriateness of this treatment quickly became apparent, reports of cases thus treated in the late 1940s and early 1950s comprise the entire body of literature on the effect of folic acid administration on persons deficient for vitamin B-12 (10–12).

Reviews of the historic case reports allude to rapid neurologic deterioration after improvement of anemia (10, 11). Consequently, the idea has developed that excessive intake of folic acid can obscure or mask vitamin B-12 deficiency and potentially delay its diagnosis until neurologic consequences become irreversible (13). Although some have questioned the connection between masking and either the diagnosis of vitamin B-12 deficiency or neurologic damage (14–18), Chodos and Ross (10) described the neurologic deterioration that occurred after folic acid administration as ''explosive'' and suggested that folic acid might actually exert a detrimental effect on the central nervous system. Furthermore, Israels and Wilkinson (11) wrote that ''some patients who had not previously shown signs of nervous system disturbances developed such signs, often very acutely, after being treated with folic acid for variable periods'' (p 1072).

Food folic acid fortification and the debate on folic acid safety

The move by the United States and other governments to fortify staple food products with folic acid for the prevention of neural tube defects rekindled the debate over the safety of folic acid for the elderly, who are at high risk of vitamin B-12 deficiency. On the basis of the historic case reports, as well as the known metabolic interaction between folate and vitamin B-12, and animal data showing a link between folate supplementation and rapid development of neuropathology in animal deficient for vitamin B-12, the Food and Nutrition Board of the Institute of Medicine called the evidence for precipitation or exacerbation of the neurologic damage of vitamin B-12 deficiency by excess supplemental folate intake ''suggestive'' and selected that endpoint, which was associated with intakes $\geq$ 5 mg/d, as the critical endpoint for the development of a tolerable upper intake for folate (13). Division of 5 mg/d by an uncertainty factor of 5 resulted in an upper intake of 1 mg/d. The high degree of uncertainty led some to question the safety of fortification (19, 20) and inspired others to criticize the upper intake as too conservative (21, 22). Particularly in light of continued demands for a higher level of fortification (23, 24), clearly defining the benefits and risks of high folic acid intake assumes vital importance. Writing about this challenge, Moore (25) observed that the current fortification program provides the perfect opportunity for epidemiologic study of the unanswered questions, including those related to the interaction between folate and vitamin B-12.

Folate status and intake in the era of folic acid fortification

Mandatory folic acid fortification of enriched cereal grain products sold in the United States officially took effect in January 1998 (26). Not surprisingly, the first report on the program's effect showed that fortification resulted in a marked increase in folate status (27, 28). Specifically, mean plasma folate increased from 4.6 ng/mL (11 nmol/L) before fortification to 10.0 ng/mL (23 nmol/L) after fortification ($P < 0.001$). Furthermore, the prevalence of folate deficiency decreased from 22.0% to 1.7%.

Although the mandated fortification of 140 μg folic acid/100 g flour was estimated to increase folic acid intake by $\approx$100 μg/d (26), our estimates (29) and those of others (30) suggest that the actual increase in intake from fortification was about twice that high. Furthermore, because of the combined intake of fortified cereal grain products, vitamin supplements, and voluntarily fortified breakfast cereals, a substantial proportion of Framingham Offspring Cohort members consumed >1 mg folic acid/d. Indeed, 11% of supplement-using cohort members and $\approx$0.3% of nonusers exceeded the upper intake amount (29).

To test the hypothesis that the high folate status achievable in the era of mandatory folic acid fortification affects the consequences of vitamin B-12 deficiency, either by a masking phenomenon or by exacerbating neurologic effects, we used data collected in recent versions of the National Health and Nutrition Examination Survey (NHANES) to evaluate the interaction between high serum folate and low vitamin B-12 status in relation to several outcomes linked to vitamin B-12 deficiency; namely, anemia, macrocytosis, cognitive impairment, elevated homocysteine concentrations, and elevated MMA concentrations (31, 32). We review and discuss the results of these studies here.

METHODS

We used data collected in the NHANES conducted during the postfortification years 1999–2002. Senior survey participants (ie, those aged $\geq$60 y), the only group whose cognitive function was assessed, numbered 3706. Of the 1684 subjects who met eligibility requirements, complete data for analyses pertaining to anemia were available for 1458 subjects, and complete data for analyses pertaining to cognitive function were available for 1302 subjects. Demographic and health data were collected by interview, and cognitive function was assessed by using a version of the Digit Symbol-Substitution subtest of the Wechsler Adult Intelligence Scale III—a screening test designed to detect cognitive impairment in adults and children (33). Blood samples

were drawn and analyzed for biochemical markers, and a complete blood count was performed.

We defined low vitamin B-12 status as serum vitamin B-12 < 148 pmol/L or serum MMA > 210 nmol/L [ie, above the published reference range for serum vitamin B-12–replete survey participants with normal serum creatinine concentrations (34)]. We defined anemia according to criteria from the World Health Organization [ie, hemoglobin < 12 g/dL for women and < 13 g/dL for men (35)], and macrocytosis as a mean cell volume > 99 fL. Finally, we used distribution-based cutoffs to define high serum folate (ie, ≥59 nmol/L, the 80th percentile) and cognitive impairment (ie, Digit Symbol-Substitution subtest score < 34/133, the 20th percentile).

RESULTS AND DISCUSSION

Interaction between vitamin B-12 status and serum folate in relation to anemia and cognition in NHANES 1999–2002

Data displayed in **Table 1** summarize the interaction between serum folate and vitamin B-12 status in relation to anemia and cognitive impairment as seen in NHANES 1999–2002 (31) and address the potential role of homocysteine in the associations. The tabulated data show that having low vitamin B-12 status, regardless of serum folate, was associated with a significantly increased prevalence of both anemia and cognitive impairment. Furthermore, compared with having normal status for both vitamins, having high serum folate but normal vitamin B-12 status was associated with a reduced prevalence of anemia and a significantly reduced prevalence of cognitive impairment. The worst combination was low vitamin B-12 status and high serum folate. Specifically, anemia and cognitive impairment were observed ≈5 times as often in the group with that combination as in the group with normal vitamin B-12 status and normal serum folate. That the odds ratios were only modestly affected by controlling for homocysteine concentrations suggests that the hematologic and cognitive risks and benefits were not directly affected by homocysteine increases or decreases.

These data represent important epidemiologic evidence of an adverse interaction between high folate status and low vitamin B-12 status. Consequently, they seem to support the idea that the neuropsychiatric consequences of vitamin B-12 deficiency are exacerbated by high folate status. We also noted with interest that, in the Nurses' Health Study, although multivitamin users in the highest plasma vitamin B-12 quartile category preformed somewhat better on cognitive function tests than did subjects who did not use supplements and were in the lowest quartile category for plasma vitamin B-12 (mean difference in global cognitive score: −0.18; 95% CI: −043, 0.07), multivitamin users who were, nevertheless, in the lowest vitamin B-12 quartile category performed worse than the comparison subjects (mean difference in global cognitive score: 0.19; 95% CI: −043, 0.07) (21). Because folic acid is a normal constituent of multivitamin pills, these findings may also reflect the adverse interaction between high folate status and low vitamin B-12 status in relation to cognition.

Our observation of a higher prevalence of not only cognitive impairment but also anemia in association with high folate status among American seniors deficient for vitamin B-12 suggests that the interaction is a general biological phenomenon (ie, that it affects all health consequences of vitamin B-12 deficiency). This finding also seems to be supported by a report from Pune, India, of a higher prevalence of hyperglycemia (which was associated with maternal vitamin B-12 deficiency) among children born to mothers deficient for vitamin B-12 with high folate status than among children born to mothers deficient for vitamin B-12 with lower folate status (20).

Interaction between circulating vitamin B-12 and folate concentrations in relation to circulating concentrations of the 2 indicators of vitamin B-12 function, homocysteine and MMA

In this study of the general US population in the age of folic acid fortification (32), we found that homocysteine does not generally decrease with increasing folate status among people with low serum concentrations of vitamin B-12 (**Table 2**). Specifically, although geometric mean homocysteine and geometric mean MMA decreased significantly across increasing serum folate categories among survey participants with serum vitamin B-12 ≥ 148 pmol/L ($P < 0.001$), the opposite trends were observed for subjects with serum vitamin B-12 < 148 pmol/L. Furthermore, geometric mean homocysteine for subjects with low serum vitamin B-12 and serum folate < 19.3

TABLE 1
Interaction between vitamin B-12 status and serum folate in relation to anemia and cognitive impairment among nonexcluded senior participants in the National Health and Nutrition Examination Survey (1999–2002) (31)

	Vitamin status						
Outcome	B-12[1]	Folate[2]	No. of Subjects	Percentage with outcome	OR (95% CI)	OR (95% CI)	OR (95% CI)
Anemia	Normal	Normal	913	3.5	1.0	1.0	1.0
	Normal	High	198	2.5	0.5 (0.2, 1.8)	0.6 (0.2, 2.5)	0.7 (0.2, 2.5)
	Low	Normal	297	6.9	1.6 (1.02, 2.6)	2.1 (1.1, 3.8)	1.9 (0.99, 3.7)
	Low	High	49	15	5.1 (2.1, 8.0)	5.2 (2.5, 11.0)	5.0 (2.4, 10.6)
Cognitive impairment	Normal	Normal	826	18	1.0	1.0	1.0
	Normal	High	180	11	0.4 (0.2, 0.7)	0.4 (0.2, 0.9)	0.5 (0.2, 0.96)
	Low	Normal	253	25	1.6 (0.99, 2.4)	1.7 (1.01, 2.9)	1.5 (0.9, 2.5)
	Low	High	42	45	4.3 (2.3, 8.0)	5.1 (2.7, 9.5)	4.7 (2.5, 8.7)

[1] Low serum vitamin B-12 < 148 pmol/L or serum methylmalonic acid above reference range (ie, 60–210 nmol/L) for vitamin B-12–replete participants with normal serum creatinine.

[2] High serum folate > 59 nmol/L (80th percentile).

TABLE 2
Least-square geometric mean (95% CI) homocysteine and methylmalonic acid concentrations by serum vitamin B-12 and serum folate category, National Health and Nutrition Examination Survey 1999–2002[1]

	Homocysteine			Methylmalonic acid		
	Serum vitamin B-12			Serum vitamin B-12		
Serum folate (nmol/L)[2]	<148 pmol/L	≥148 pmol/L	*P*	<148 pmol/L	≥148 pmol/L	*P*
16	9.9 (8.8, 11.1)	9.2 (9.0, 9.4)	0.222	174 (149, 204)	138 (133, 145)	0.005
23	10.8 (9.4, 12.4)	8.0 (7.8, 8.2)	<0.001	265 (218, 322)	137 (132, 142)	<0.001
30	10.5 (9.1, 12. 0)	7.5 (7.4, 7.7)	0.001	265 (194, 363)	132 (127, 137)	<0.001
44	11.8 (10.1, 13.8)	7.1 (7.0, 7.2)	<0.001	314 (234, 421)	128 (125, 130)	<0.001

[1] Values were generated with the use of SUDAAN PROC REGRESS (Research Triangle Institute, Research Triangle Park, NC) after controlling for age, race-ethnicity, sex, cigarette smoking, alcohol intake, BMI, self-reported diabetes status, and serum concentrations of creatinine and alanine aminotransferase.

[2] Values in this column are category medians.

nmol/L was in the normal range and close to that of subjects in the same serum folate category with higher serum vitamin B-12. However, the mean for the group with low serum vitamin B-12 and serum folate > 32.6 nmol/L was nearly 12 μmol/L, a commonly used cutoff for hyperhomocystinemia, and serum MMA was extremely elevated.

In vitamin B-12 deficiency, high homocysteine reflects impaired MS activity, whereas high MMA indicates impaired methylmalonyl-CoA mutase activity. Consequently, our findings suggest that both pathways of vitamin B-12 metabolism are adversely affected by high serum folate despite the direct involvement of folate only in MS activity. Simultaneous impairment of both pathways is normally caused by insufficient intake of vitamin B-12 or by the disruption of early steps in vitamin B-12 processing (1, 23). The latter include intrinsic factor-mediated intestinal absorption, formation of the vitamin B-12–transcobalamin II complex in plasma, transport of the complex into peripheral tissue by a receptor-mediated endocytosis, incorporation of the complex into lysosomes, dissociation of the complex and exit of vitamin B-12 as acquocob(III)alamin into the cytosol, and reduction to cob(II)alamin for subsequent distribution to MS in the cytosol or to the mitochondria. The event that effects the simultaneous disruption of both pathways could also occur after the distribution of vitamin B-12 to the respective compartments in the course of rendering the vitamin available for interaction with the respective enzyme proteins or during catalysis.

We found no adverse interactions between high serum folate and low vitamin B-12 status among participants of the NHANES III, which took place in 1991–1994, before mandatory food folate fortification took effect (19). Although most of the seniors with serum folate concentrations > 59 nmol/L who participated in the 1999–2002 NHANES achieved their high folate status through the combined intake of enriched cereal grain products, fortified breakfast cereals, and multivitamins, the influence of fortification is indicated by the 40% prevalence of serum folate > 59 nmol/L among supplement-using seniors who participated in the 1999–2002 NHANES, compared with a 10% prevalence among comparable participants in the NHANES III. Note that most subjects with high folate status consumed supplemental vitamin B-12 and absorbed it. Indeed, mean vitamin B-12 concentration (95% CI) for senior 1999–2002 NHANES participants with low vitamin B-12 status and high serum folate was 354 pmol/L (95% CI: 309, 406 pmol/L) compared with 242 pmol/L (95% CI: 228, 257 pmol/L) for the subgroup with low vitamin B-12 status and normal serum folate. Although unpublished results of our analyses of Framingham Study data showed that 80% of the folate ingested by people with circulating folate concentrations > 50 nmol/L was in the form of folic acid, it remains to be determined whether the interaction we observed is a unique consequence of circulating unmetabolized folic acid or merely the result of a high total (ie, both reduced and oxidized) mass of body folate (Other articles in this supplement to the Journal include references 36–39).

The authors' responsibilities were as follows—JS: originated the idea and reviewed the manuscript; MSM: performed statistical analyses and prepared the manuscript; PFJ: reviewed statistical results and contributed to the manuscript; and IHR: discussed ideas and reviewed the manuscript. None of the authors had a conflict of interest.

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